510(k) Safety and Effectiveness Summary

Submitted by:

Immunetics, Inc.
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Cambridge, MA 02142

Contact Person:

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Date of Preparation:

December 4, 2000

1. **Name and Address of Owner/Operator and Manufacturer**

   Immunetics, Inc.
   63 Rogers Street
   Cambridge, MA 02142

2. **Product Name**

   Trade Name: Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit

   Common Name: *B. burgdorferi* IgG/IgM ELISA Kit

3. **Claim of Substantial Equivalence**

   The characterized samples used for the establishment of Substantial Equivalence were obtained from patients with a clinical diagnosis of Lyme Disease in accordance with the CDC case definition, i.e. based on the presence of EM (erythema migrans) or the presentation of late Lyme clinical manifestations (e.g., arthritic, cardiac, or neurological symptoms). Infection was confirmed by culture of *Borrelia* from biopsies in many examples.

   The Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit is substantially equivalent to the predicate device based upon the assessment of performance of the kit in clinical trials in which well characterized, archived Lyme Disease specimens, Centers for Disease Control Lyme Disease Serum Panel, normal donor specimens (from endemic

and non-endemic regions), samples from diverse disease conditions and samples from individuals who have received the licensed recombinant OspA Lyme disease vaccine (Lymerix®, manufactured by GlaxoSmithKline Biologicals) were analyzed.

4. Description

The antigen used in the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit is a synthetic peptide derived from the VlsE protein, which has been shown to be both specific and highly immunogenic. As the antigen represents a defined sequence within the protein, potential cross-reactivity with unrelated and partially related antigens found in other organisms is greatly reduced. Likewise, cross-reactivity in individuals vaccinated with the licensed OspA vaccine against Lyme disease (Lymerix®, manufactured by GlaxoSmithKline Biologicals) is not observed.

The Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit is based on a synthetic peptide antigen in microwell ELISA format. In the assay procedure, diluted serum samples are added to and incubated in wells of an antigen-coated microwell plate. Antibodies specific to the VlsE peptide in the serum sample are bound by the immobilized antigen, and unbound antibodies are removed by wash steps. The bound antibodies are detected by addition of a horseradish peroxidase-conjugated (HRP) goat anti-human IgG/IgM conjugate. After removal of excess conjugate by further wash steps, a chromogenic peroxidase substrate containing tetramethylbenzidene is added. A blue-green product is produced in wells where antibodies have been bound to the antigen. The color development reaction is quenched by addition of dilute sulfuric acid, after which optical absorbance at 450 nm is measured in each well using an ELISA microplate reader.

5. Intended Use

The Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit is intended for use in the presumptive detection of IgG and IgM antibodies to *B. burgdorferi* in human serum. The assay should be used only on samples from patients with clinical history, signs or symptoms consistent with *B. burgdorferi* infection, including individuals who have received the licensed recombinant OspA Lyme disease vaccine (Lymerix®†). Positive or equivocal results should be supplemented by testing with a standardized Western Blot (second step) method. Positive Western Blot results provide evidence for exposure to or infection with *B. burgdorferi*. The diagnosis of Lyme disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi*. Negative results (either first or second step) should not be used to exclude Lyme disease.

† Lymerix® is a registered trademark of GlaxoSmithKline Biologicals.

6. Summary of Performance

From a summary of the clinical trial data, the following performance characteristics are described:

Expected values

Three (3) investigational sites, including Immunetics and two (2) independent off-site investigators, assayed samples from the following patient populations:

1. Normal, healthy individuals (n=197) comprised of samples from Lyme disease endemic (n=99) and non-endemic (n=98) regions.

2. Individuals vaccinated with a licensed OspA vaccine against Lyme disease (Lymerix®, manufactured by GlaxoSmithKline Biologicals) (n=43).

3. Lyme disease panel (n=180) compromised of samples from individuals with a clinical diagnosis of Lyme disease, including (n=141) sera which were positive by ELISA and Western Blot assays and (n=39) sera in a panel provided by the Centers for Disease Control (CDC).

4. A prospective study (n=191) on samples obtained from individuals whose sera were sent to a reference laboratory for Lyme screening tests.

Reproducibility

Reproducibility was tested on a panel of 9 specimens, including the kit Positive, Negative and Calibrator controls and 6 specimens representing 2 negative, 2 weakly reactive and 2 positive sera. Reproducibility was assessed in four analyses, intra-assay, inter-assay, inter-lot and inter-site.

The Intra-Assay Reproducibility was determined by testing 9 specimens in 10 wells of one ELISA plate derived from one Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit at Immunetics. The average coefficient of variation was 9.5, with a range from approximately 5 to 16. The results indicate that reproducible test results are obtained in multiple ELISA plate wells within a single assay run of the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit.

The Inter-Assay Reproducibility was determined by testing 9 specimens in 10 wells in each of 3 ELISA plates derived from 3 separate Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kits manufactured in a single lot. The coefficient of variation ranged from approximately 4 to 26, with an average value of 14.3. Results thus show that reproducible values are generated when specimens are tested in independent assay runs using the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit .

The Inter-Lot Reproducibility was determined by testing 9 specimens in 10 wells of each of 3 ELISA plates derived from three separate manufactured lots of the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit. The average coefficient of variation across all samples and all lots was 9.75, within a range of approximately 6 to 13, indicating that the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit yields reproducible results from lot to lot.

The Inter-Site Reproducibility was determined by testing 9 specimens in 10 wells of Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit plates derived from a single lot at

each of the three sites. The average coefficient of variation across all samples was 16.47, with a range of values from approximately 7 to 26. These results indicate that the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit performs reproducibly when tested at different sites.

Reactivity in Normal Population

One hundred ninety seven (197) serum specimens were obtained from normal blood donors, comprising 99 sera from individuals residing in regions endemic for Lyme disease (northeastern U.S.) and 98 sera from individuals residing in areas considered non-endemic for Lyme disease (southwestern U.S.). Sera were tested once on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit and the Wampole *B. burgdorferi* ELISA. The reactivity of the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit was less than or equal to that of the predicate device for each category of normal specimens tested, while the overall reactivity of 2% for endemic and non-endemic specimens combined was lower than that obtained with the Wampole *B. burgdorferi* kit. Furthermore, LI values produced by the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ were distributed over a range which was both narrower and, with respect to the cut-off, lower than the comparable ISR values produced by the Wampole ELISA, resulting in fewer equivocal results (1 out of 197, or 0.5% for the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit, 7 out of 197 or 3.5% for the Wampole *B. burgdorferi* ELISA). The Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit thus offers low reactivity, a low frequency of equivocal results, and does not generate a significant number of positive results in an average, healthy population.

	Endemic	Non-Endemic	Total	95% CI
Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit	3%	1%	2%	1-5%
Wampole *B. burgdorferi* ELISA	3%	8%	6%	3-10%
n =	99	98	197	

Reactivity in Vaccine Recipients

A panel of 50 sera was obtained comprising 43 sera from individuals who were vaccinated with the Lymerix® vaccine (recombinant OspA vaccine from GlaxoSmithKline Biologicals) and 6 controls as part of the clinical trial (protocol 014) carried out by SmithKline Beecham to support an application for licensing of the vaccine by the U.S. Food and Drug Administration. Individuals participating in the trial were negative on Lyme ELISA tests prior to the start of the trial and were in good health. Serum samples were tested on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit and on the Wampole *B. burgdorferi* Lyme ELISA. From these results it is evident that the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ is non-reactive with sera from individuals vaccinated with a licensed recombinant OspA vaccine, while the Wampole *B. burgdorferi* ELISA exhibits 100% reactivity with the same specimens.

	Negative	Pos/Equiv	95% CI (Negative)
Immunetics® C6 *B. burgdorferi* (Lyme)ELISA™ Kit	100%	0%	93-100%
Wampole *B. burgdorferi* ELISA	0%	100%	0-7%
n = 43			

Sensitivity

In Retrospective studies serum samples from 180 patients with clinical diagnoses of Lyme disease were tested on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit and on the Wampole *B. burgdorferi* ELISA kit. The overall sensitivity reported for the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ is slightly higher than that of the Wampole ELISA, indicating that the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit can be used as a sensitive test for exposure to *B. burgdorferi*.

	Sensitivity	95% CI
Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit	97%	93-99%
Wampole *B. burgdorferi* ELISA	94%	89-97%
n = 180		

In Prospective studies serum samples from 191 patients whose sera were sent to a reference laboratory for Lyme screening tests were tested at Immunetics and a second site (BBI) on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit and the Wampole *B. burgdorferi* ELISA kit. Concordant results were found in 77.5% of the specimens tested. Of the 22.5% discrepant results, 22.0% represented specimens found positive or equivocal on the Wampole *B. burgdorferi* ELISA and negative on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit. One-half percent of the specimens were found negative on the Wampole *B. burgdorferi* ELISA and positive or equivocal on the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit.

Of the 43 prospective serum samples yielding discrepant results in the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ vs. Wampole ELISA, a subset of 23 samples was available for further testing by Western Blot. Western Blot testing was performed using the 510(k)-cleared Immunetics QualiCode *B. burgdorferi* IgG and IgM Western Blot kits. Results indicate that the majority (19, or 83%) of sera tested, which were negative by Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ but positive by Wampole ELISA, were negative by Western Blot. The 2 sera which were negative by Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ and positive by Western Blot included 1 serum which was positive by IgM Western Blot alone, and 1 serum which was positive by IgG Western Blot alone. These may possibly represent either very early Lyme infections or non-specific Western Blot reactivity.

Thus, the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit provides substantially

more accurate results than the Wampole ELISA when compared with Western Blot in testing prospective patient samples. The greater level of accuracy of the Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ in the testing of prospective samples generates fewer false positive results, thereby resulting in a decrease in the number of samples requiring further Western Blot testing according to the two-tier CDC protocol.

Cross-Reactive Conditions

Sera from 178 individuals with disease conditions other than Lyme disease were tested at Immunetics. Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit results are provided for fourteen conditions as listed in the following table. Results indicate that cross-reactivity was minimal with all disease conditions tested. Those instances of cross-reactivity detected may have been due to substances causing interference in the assay, actual antigenic cross-reactivity, or possible exposure to *B. burgdorferi*.

Disease Condition	n	Pos/Equiv
AntiNuclear Antibodies	18	1
Bilirubinemic	5	0
H. pylari	18	0
Hemolyzed	4	1
HIV	18	0
Lipemic	5	0
Mononucleosis	17	0
Multiple Sclerosis	20	0
Periodontal	4	1
Relapsing Fever	18	0
Rheumatoid Arthritis	16	1
Systemic Lupus Erythematosus	10	1
Syphilis	20	1
Tularemia	5	0

7. Conclusions

Based on the clinical performance, this device has been shown to be safe and effective for the intended use in the presumptive detection of IgG and IgM antibodies to *B. burgdorferi* in human serum from vaccinated and unvaccinated populations.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

MAY 1 0 2001

Andrew E. Levin, Ph.D.
President and chief Executive Officer
Immunetics, Inc.
63 Rogers Street
Cambridge, MA 02142

Re: K003754
Trade Name: Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit
Regulation Number: 866.3830
Regulatory Class: II
Product Code: LSR
Dated: February 28, 2001
Received: March 1, 2001

Dear Dr. Levin:

We have reviewed your Section 510(k) notification of intent to market the device referenced above and we have determined the device is substantially equivalent to devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (Premarket Approval), it may be subject to such additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 895. A substantially equivalent determination assumes compliance with the Good Manufacturing Practice for Medical Devices: General (GMP) regulation (21 CFR Part 820) and that, through periodic GMP inspections, the Food and Drug Administration (FDA) will verify such assumptions. Failure to comply with the GMP regulation may result in regulatory action. In addition, FDA may publish further announcements concerning your device in the Federal Register. Please note: this response to your premarket notification submission does not affect any obligation you might have under sections 531 through 542 of the Act for devices under the Electronic Product Radiation Control provisions, or other Federal laws or regulations.

This letter will allow you to begin marketing your device as described in your 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific advice for your device on our labeling regulation (21 CFR Part 801 and additionally 809.10 for in vitro diagnostic devices), please contact the Office of Compliance at (301) 594-4588. Additionally, for questions on the promotion and advertising of your device, please contact the Office of Compliance at (301) 594-4639. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its internet address "http://www.fda.gov/cdrh/dsma/dsmamain.html".

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Division of Clinical Laboratory Devices
Office of Device Evaluation
Center for Devices and Radiological Health

Enclosure

510(k) Number (if known): K003754

Device Name: Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit

Indications For Use:

The Immunetics® C6 *B. burgdorferi* (Lyme) ELISA™ Kit is intended for use in the presumptive detection of IgG and IgM antibodies to *B. burgdorferi* in human serum. The assay should be used only on samples from patients with clinical history, signs or symptoms consistent with *B. burgdorferi* infection, including individuals who have received the licensed recombinant OspA Lyme disease vaccine (Lymerix®, manufactured by GlaxoSmithKline Biologicals). Positive or equivocal results should be supplemented by testing with a standardized Western Blot (second step) method. Positive Western Blot results provide evidence for exposure to or infection with *B. burgdorferi*. The diagnosis of Lyme disease must be made based on history, signs (such as erythema migrans), symptoms, and other laboratory data, in addition to the presence of antibodies to *B. burgdorferi*. Negative results (either first or second step) should not be used to exclude Lyme disease.

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Concurrence of CDRH, Office of Device Evaluation (ODE)

Woody Dubois
(Division Sign-Off)
Division of Clinical Laboratory Devices
510(k) Number K 003754

Prescription Use ✓ OR Over-The-Counter Use ____
(Per 21 CFR 801.109)

(Optional format 1-2-96)